Protective Effect of Anisodamine on Brain Edema Induced by Pertussis Bacilli in Rabbits

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ABSTRACT

Objective: To observe the effect of 654-2 on brain edema model induced by pertussis bacilli in rabbits. Methods: Brain edema model of 26 rabbits induced by pertussis bacilli was prepared for observation, their brain water content and brain glutamate level were determined before and after treatment. Results: 654-2 could decline the water content of brain and increase the glutamate level in brain tissue of the treatment group as compared with group without treatment. Conclusion: 654-2 has protective effect on brain edema induced by pertussis bacilli.

KEY WORDS anisodamine, bordetella pertussis, brain edema models, glutamates

Anisodamine (654-2) was a kind of artificially synthesized herbal medicine with its action quite similar to atropine, but less side effect. It was widely and effectively used in treating septic shock but its effect on infectious brain edema has not been reported. In order to find a new way of treating infantile brain edema, the effect of 654-2 on experimental mixed type brain edema induced by pertussis bacilli (PB) was observed in this study.

METHODS

Animals
Twenty-six rabbits, 1.5 ~ 2.0 kg in weight, were divided into three groups: 7 in the control group, 10 in the untreated model group and 9 in the 654-2 treated group.

Experiment
Animal model was prepared according to the method of the author's laboratory(1), 0.6 ml/kg of PB suspension 5 x 10^10PB/ml in concentration was injected into the right internal carotid artery of rabbit to induce brain edema and 2.5% Even's blue (1ml/kg) was also given intravenously for indicating breakdown of blood-brain barrier. To control group, equal volume of normal saline was injected instead of PB suspension.

To treated group, 5 mg/kg of 654-2 was intravenously injected immediately after the PB injection. Then, 2.5 mg/kg of 654-2 was given intravenously every thirty minutes for 11 doses. The animals were decapitated 6 hours after PB injection with their bilateral cerebral hemispheres removed rapidly.

Brain Water Content Determination
One to two hundred milligram of fresh brain tissue was dried at 105°C for 48 hours to constant weight (the difference the 2 last weights being ≤0.2 mg)(2). Wet-dry weight measurement was used to show the water content in brain tissue.

Brain Glutamate Level Determination
One ml alcohol was added in 100 mg of brain tissue to homogenize in an ice box and the homogenate was centrifuged under 4°C for 20 minutes (18000 rpm). Content of glutamate in the supernate was determined by high performance liquid chromatography(3).

Statistical Analysis
Data between two groups were analyzed by t-test or rank sum test and paired t-test was adopted for comparison between right and left brain of the same animal.
RESULTS

Brain Water Content (BWC)

The average BWC of both hemispheres of the untreated model group was significantly higher than that of the control group (left $P < 0.05$, right $P < 0.01$) and its right BWC was higher than left BWC ($P < 0.01$).

The right BWC of the 654-2 treated group was lower than that of the untreated group ($P < 0.05$) but higher than that of the control group ($P < 0.05$). Its left BWC was less than the right ($P < 0.01$). There was no significant difference of left BWC between the 654-2 treated group and the control group ($P > 0.05$).

Brain Glutamate Level (BGL)

The average right BGL in either untreated or treated model groups was lower than the left BGL. And it was higher in 654-2 treated group than in untreated group (see table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BWC (%)</th>
<th>n</th>
<th>BGL (μmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>78.80 ± 0.77</td>
<td>79.18 ± 0.65</td>
<td>5</td>
</tr>
<tr>
<td>Untreated</td>
<td>10</td>
<td>80.46 ± 1.31*</td>
<td>82.75 ± 1.52**</td>
<td>7</td>
</tr>
<tr>
<td>654-2 treated</td>
<td>9</td>
<td>79.79 ± 1.18</td>
<td>81.00 ± 1.20**</td>
<td>6</td>
</tr>
</tbody>
</table>

Notes: * $P < 0.05$, ** $P < 0.01$, compared with control group; *' $P < 0.05$, compared with untreated group; *'' $P < 0.01$, compared between left and right in same group.

Even’s Blue Discoloration

The range and degree of Even’s blue discoloration between the 654-2 treated and the untreated model group was not different significantly (see table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Range</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>I</td>
</tr>
<tr>
<td>Untreated</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>654-2 treated</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0</td>
</tr>
</tbody>
</table>

Notes: Range = 0 no, I <1/4 hemisphere, II 1/4–1/2 hemisphere, III >1/2 hemisphere; Degree = 0 no, + light blue, ++ blue, +++ dark blue

DISCUSSION

Glutamate is an important excitatory neurotransmitter which exists mainly in the neuron. When intracellular stores of glutamate are released into the extracellular space, the concentration of glutamate in interstitial tissue of brain increases. The toxic effect of glutamate can produce both immediate neuronal swelling caused by entry of sodium chloride and water into the cell, and late neuronal damage by way of calcium accumulation in the cell. In recent years many researches found that the excitatory neurotoxicity of released endogenous glutamate plays an important role in the pathogenesis of various damage of the central nervous system damage$^{(4)}$. Erecinska reported that content of glutamate in brain was significantly declined after ischemic-reperfusion due to the released glutamate being carried away by the blood$^{(5)}$. Whether glutamate acts on the pathogenesis of brain damage in PB induced brain edema has not been reported. This study showed the BWC was significantly decreased while the BGL was significantly decreased in untreated group, it suggested that the glut-